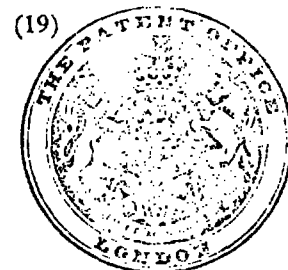


PATENT SPECIFICATION

(11) 1 461 323

1 461 323

- (21) Application No. 8097/74 (22) Filed 22 Feb. 1974
 (31) Convention Application No. 335 381
 (32) Filed 23 Feb. 1973 in
 (33) United States of America (US)
 (44) Complete Specification published 13 Jan. 1977
 (51) INT CL³ C07D 501/60; A61K 31/545
 (52) Index at acceptance



C2C 1314 1470 1510 200 214 215 220 226 227 22Y 246 247
 253 254 256 25Y 28X 292 29X 29Y 30Y 311 313 314
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(72) Inventor ROBERT RAYMOND CHAUVETTE

(54) ALPHA-AMINOACYL-3-HALO CEPHALOSPORINS

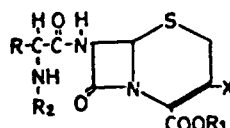
(71) We, ELI LILLY AND COMPANY, a corporation of the State of Indiana, United States of America, having a principal place of business at 307 East McCarty Street, City of Indianapolis, State of Indiana, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to 7- α -aminoacyl-3-halo cephalosporins and the non-toxic pharmaceutically acceptable salts thereof, valuable, orally effective antibiotics.

Several 7- α -aminoacyl cephalosporin antibiotics with varying substituents in the 3-position of the molecule are known. For example, the well-known antibiotic cephalixin, 7-(D- α -phenylglycylamido)-3-methyl-3-cephem-4-carboxylic acid, a desacetoxyccephalosporanic acid which has a methyl group in the 3-position; the antibiotic cephaloglycine, 7-(D- α -phenyl-glycylamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid, a cephalosporanic acid having an acetoxymethyl group in the 3-position; and the α -aminoacyl cephalosporins wherein the 3-position is substituted by a thiadiazolthiomethyl or tetrazolthiomethyl moiety are all valuable therapeutic agents. Also a number of cephalosporin compounds have been described wherein the molecule is substituted in the 3-position by a 3-halomethyl group, for example, 3-bromomethyl-3-cephem-4-carboxylic acid esters. The 3-halomethyl cephalosporin esters which have been described are disclosed as useful intermediates for the preparation of cephalosporin antibiotics.

The halo cephalosporin compounds provided by this invention are structurally unique compounds of the cephalosporin class in that the halogen atom is attached directly to the carbon in the 3-position of the dihydrothiazine ring. In contrast, previous halo derivatives of cephalosporins, such as those described above, bear a halogen atom on a methylene group attached to the carbon atom in the 3-position of the dihydrothiazine ring. The novel compounds thus provided have a combination of features which comprise a 7- α -aminoacyl moiety attached to a cephalosporin nucleus which is substituted in the 3-position with a fluoro, chloro, or bromo atom.

The 7- α -aminoacyl-3-halo cephalosporin compounds of the invention are represented by the following formula I



wherein R is phenyl, monohydroxyphenyl, dihydroxyphenyl, monohalophenyl, dihalophenyl, monomethylphenyl, dimethylphenyl, monomethylphenyl, dimethoxyphenyl, 2-thienyl, 3-thienyl or 2-furyl;

SPECIFICATION AMENDED - SEE ATTACHED SHEET

R_1 is hydrogen, benzyl, p-methoxybenzyl, p-nitrobenzyl, diphenylmethyl, 2,2,2-trichloroethyl, trimethylsilyl, or t-butyl;

R_2 is hydrogen or an amino protecting group;

X is fluoro, chloro, or bromo;

5 and when R_1 or R_2 is hydrogen the pharmaceutically acceptable non-toxic salts thereof.

Preferably, R_2 is a hydrogen atom.

10 Mono and dihydroxyphenyl groups are, for example, 4-hydroxyphenyl, 3-hydroxyphenyl, 2-hydroxyphenyl, 3,4-dihydroxyphenyl, and 2,4-dihydroxyphenyl. Mono and di halo substituted phenyl groups wherein halo refers to fluoro, chloro and bromo, are, for example, 4-fluorophenyl, 4-chlorophenyl, 2,4-dichlorophenyl, 3-chlorophenyl, 2-chlorophenyl, 3-bromophenyl, or 4-bromophenyl. Mono methylphenyl groups are for example the isomeric 2, 3, and 4-methylphenyl groups and the dimethylphenyl groups are, for example, 3,4-dimethylphenyl, and 2,4-di-

15 methylphenyl. Mono and di methoxyphenyl groups are, for example, 4-methoxyphenyl, 3-methoxyphenyl, 2-methoxyphenyl, 3,4-dimethoxyphenyl, and 2,6-dimethoxyphenyl.

20 Preferably, R is phenyl. Further embodiments of the invention comprise compounds of formula I wherein (a) R is mono or dihydroxyphenyl; (b) R is mono dihalophenyl; or (c) R is 2-thienyl.

The ester groups represented by R_1 in the above formula are all known ester moieties which are commonly employed in the cephalosporin art to protect the C_4 carboxyl group of the cephalosporin molecule while reactions involving other reactive sites in the molecule are carried out.

25 The term, "pharmaceutically acceptable non-toxic salts", refers to both the salts of the C_4 carboxylic acid function and the acid addition salts of the α -amino group of the 7-glycylamido side chain. Pharmaceutically acceptable salts involving the C_4 carboxylic acid function include the salts formed with inorganic bases such as the sodium, potassium and calcium salts which can be prepared with sodium bicarbonate, potassium carbonate, calcium hydroxide, or sodium hydroxide. Pharmaceutically acceptable amine salts can also be prepared for example with the organic amines such as dicyclohexylamine, benzylamine, 2-aminoethanol, diethanolamine, or diisopropylamine. Acid addition salts of the α -amino group include the salts formed with the mineral acids such as hydrochlorides, hydro-

35 bromides and the sulfates and the salts formed with organic sulfonic acids such as the p-toluenesulfonates.

It will be readily recognized that when R_1 and R_2 are hydrogen the zwitterionic form of the compounds can exist which form arises via intramolecular salt formation.

40 Owing to the presence of the asymmetric carbon atom in the α -aminoacyl group the 3-halo cephalosporins of the above formula include the D-, L- and DL- forms. The D- form is the preferred isomeric form of the present invention.

Examples of the α -aminoacyl-3-halo-cephalosporins represented by the above formula are the following:

45 7-(D- α -phenylglycylamido)-3-fluoro-3-cephem-4-carboxylic acid,

7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid,

7-(D- α -phenylglycylamido)-3-bromo-3-cephem-4-carboxylic acid,

50 7-[D- α -(4-hydroxyphenyl)-glycylamido]-3-fluoro-3-cephem-4-carboxylic acid,

7-[D- α -(3-hydroxyphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid,

7-[D- α -(2-thienyl)glycylamido]-3-chloro-3-cephem-4-carboxylic acid,

55 7-[D-2-(2-furyl)glycylamido]-3-chloro-3-cephem-4-carboxylic acid,

7-[D- α -(4-chlorophenyl)-glycylamido]-3-bromo-3-cephem-4-carboxylic acid,

7-[D- α -(3-methylphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid,

60 7-[D- α -(2,6-dimethoxyphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid,

7-[D- α -(3-thienyl)-glycylamido]-3-fluoro-3-cephem-4-carboxylic acid,

7-[D- α -(3-thienyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid,

as the zwitterionic forms and the pharmaceutically acceptable salts thereof.

65 The term " α -" in connection with the compounds listed above, and other phenyl derivatives of the invention, indicates the portion of attachment of the

2	3	1,461,323	3.
dimethyl.		phenyl group, and does not refer to stereochemical features of the molecule.	
		The compounds of the formula I wherein R ₁ is hydrogen or the pharmaceutically acceptable non-toxic salts thereof are valuable antibiotics useful for combating infections in warm blooded mammals caused by gram-positive and gram-negative microorganisms. They are effective when administered parenterally, for example, subcutaneously or intramuscularly, as well as when administered orally.	
carboxylic salts	5	5	5
phenyl, 3-phenyl, 3-chloro-phenyl, Mono-yl groups and 2,4-dimethoxy-2,6-di-	10	10	10
	15	15	15
comprise R is mono	20		
own ester ect the C ₄ ing other			
both the e α-amino involving bases such th sodium hydroxide. mple with thanol, di-ino group es, hydro-ids such as	25		
rogen the via intra-			
acyl group DL- forms.	40		
the above	45		
cephem - 4-			
cephem - 4-	50		
cephem - 4-			
xylic acid, xylic acid, cephem - 4-	55		
- carboxylic	60		
- carboxylic			
thereof, and other ment of the	65		
	20		20

Table I.

(7-(D-α-Phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid <i>in vitro</i> spectrum	
Organism*	MIC (μg./ml.)
<i>Staphylococcus aureus</i> 3055	1
<i>Staphylococcus aureus</i> 3074	1
<i>Streptococcus faecalis</i> X66	16
<i>Proteus morganii</i> PR15	>128
<i>Salmonella typhosa</i> SA12	<.5
<i>Klebsiella pneumoniae</i> KL14	.2
<i>Enterobacter aerogenes</i> EB17	8
<i>Serratia marcescens</i> SE3	>128
<i>Escherichia coli</i> EC14	2
<i>Citrobacter freundii</i> CF17	>128
<i>Pseudomonas aeruginosa</i> X239	>128
<i>Bordetella bronchiseptica</i> 16	64
<i>Salmonella typhimurium</i>	1
<i>Pseudomonas solanacearum</i> X185	<128
<i>Erwinia amylovora</i>	1

*Numbers and/or letters following the organism name refer to strains.

Table II below lists the diameter in millimeters of the zone of inhibition of growth of the indicated micro-organisms in the standard disc-plate assay obtained with 7 - (D - α - phenylglycylamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid.

Table II.

Microorganism	Zone of inhibition* (mm-diameter) Concentration (mg/ml)		
	1.0	0.1	0.01
<i>Staphylococcus aureus</i>	35	28	20
<i>Bacillus subtilis</i>	44	32	21
<i>Sarcina lutea</i>	48	27	27
<i>Mycobacterium avium</i>	24	Tr	—
<i>Proteus vulgaris</i>	23	Tr	—
<i>Salmonella gallinarum</i>	35	25	13
<i>Escherichia coli</i>	30	20	11
<i>Klebsiella pneumoniae</i>	28	20	12
<i>Pseudomonas solanacearum</i>	32	23	—

*Tr indicates a trace zone.

A dash (—) indicates no observed zone.

In Table III which follows the MIC value obtained with 7 - (D - α - phenyl-glycylamido) - 3 - chloro - 3 - cephem - 4 - carboxylic acid against a spectrum of gram-positive and gram-negative microorganisms in the agar dilution test is shown.

Table III.

In vitro Antibiotic Spectrum of 7-(D- α -phenylglycylamido)-3-chloro- 3-cephem-4-carboxylic acid		
Test Organism*		Minimum Inhibitory Concentration MIC μ g./ml.
<i>Staphylococcus aureus</i>	3055	1.0
do	3123	1.0
do	3074	2.0
<i>Streptococcus</i> (Group D)	9901	64
<i>Enterobacter cloacae</i>	EB9	>128
<i>Enterobacter aerogenes</i>	EB17	64
<i>Escherichia coli</i>	EC14	1.0
do	EC35	2.0
do	EC38	1.0
<i>Klebsiella</i> sp.	KL3	>0.5
do	KL14	2.0
do	KL25	2.0
<i>Proteus mirabilis</i>	PR6	1.0
<i>Proteus morganii</i>	PR1	128
<i>Proteus rettgeri</i>	PR9	>128
do	PR2	>128
<i>Salmonella</i>	SA12	<0.5
<i>Shigella</i> sp.	SH3	2.0

*Numbers and/or letters following the organism name refers to strains.

In the standard agar dilution test, 7 - (D - α - phenyl - glycylamido) - 3-chloro - 3 - cephem - 4 - carboxylic acid demonstrated activity against *Haemophilus influenzae* having MIC values of from 1 to 4 mg/ml against a number of strains.

In Table IV which follows the oral absorption of 7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid is demonstrated by the blood and urine levels obtained in mice. In carrying out the test McAllister Swiss mice weighing from 11 to 13 grams were fasted overnight and were then orally administered 20 mg./kg. of 7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid. Blood and urine samples were taken at the indicated intervals and the concentration of the antibiotic in each sample was determined by microbiological assay using *Sarcina lutea* in pH 6.0 agar in the disc-plate assay.

Table IV.
7-(D- α -Phenylglycylamido)-3-chloro-
3-cephem-4-carboxylic acid

Blood and Urine Levels

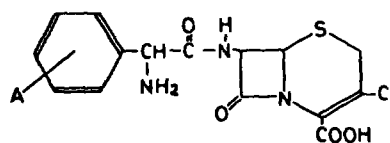
Mouse No.	Concentration $\mu\text{g./ml.}$ at time intervals (min.)							
	5	15	30	60	90	120	240	
1	29.1	16.2	11.6	3.5	1.2	0.5	0.2	
2	23.7	19.2	11.0	5.2	3.2	2.0	0.6	
3	17.6	10.1	9.1	5.7	2.1	1.3	0.7	
4	23.3	13.6	10.1	4.0	1.6	1.6	1.0	
Average Blood	23.4	14.8	10.5	4.6	2.0	1.4	0.6	
Average in Pooled urine	—	1474	1764	843	305	308	226	

The effective dose (ED_{50}) for 7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid which is illustrative of the effective dose for the compounds of the invention is 0.74 mg./kg. $\times$ 2 oral and 0.48 mg./kg. $\times$ 2 s.c. vs. *Streptococcus pyogenes*, and 5.5 mg./kg. $\times$ 2 orally vs. *Escherichia coli* and 17.6 mg/kg $\times$ 2 orally vs. *Diplococcus pneumoniae* as determined in mice.

In Table V, below, are presented the minimum inhibitory concentrations (MIC) against representative gram-negative bacteria for illustrative 3-halo-3-cephem compounds of the invention. The inhibitory concentrations were determined by the Gradient-Plate method carried out essentially as described by Brijson and Szybalski, *Science* 116, 45 (1952). In the Table V the A terms have reference to the structural formula.

Table V.

Antibiotic Activity of Substituted
7-(α -Phenylglycylamido)-3-chloro-3-cephem-
4-carboxylic acids vs. Gram-Negative
Bacteria

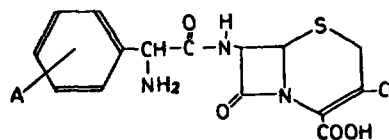


Test Organism	Minimum Inhibitory Concentration ($\mu\text{g/ml}$)				
	A	3—OH	4—OH	4—Cl	H
<i>Shigella</i> sp.		1.0	2.0	13.3	6.7
<i>Escherichia coli</i>		1.0	2.0	17.2	5.7
<i>Klebsiella pneumoniae</i>		0.9	1.5	8.0	0.9
<i>Aerobacter aerogenes</i>		0.8	1.0	5.0	0.8
<i>Salmonella heidelberg</i>		0.5	0.8	10.7	0.2
<i>Pseudomonas aeruginosa</i>		>200	>200	>200	>200

In the following Table VI, the activity of representative 3-halo-3-cephem compounds against several clinical isolates of penicillin-resistant staphylococci is presented. The activity is presented in minimum inhibitory concentrations of the test compound. The minimum concentrations were determined by the Gradient-Plate method.

Table VI.

Antibiotic Activity of Substituted
7-(α -Phenylglycylamido-3-chloro-3-
cephem-4-carboxylic acids vs.
Penicillin Resistant Staphylococci

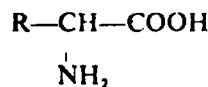


Staphylococcus ¹ Strain	Minimum Inhibitory Concentration (μ g/ml)				
	A	3-OH	4-OH	4-Cl	H
V41		3.0/	5.0/	10.5/>20	11.5/>20
V32		3.5/	7.0/	18/>20	17/>20
X400		>20/	>20/	>20/>20	>20/>20
V84		0.5/	0.6/	3.0/>20	2.7/15
X1.1		0.4/	0.6/	0.4/0.7	0.4/1.0

1/ The letter-numeral designations refer to strains of clinical isolates of penicillin-resistant staphylococcus.

2/ In the columns, the value above the slash is the MIC in the absence of human serum. The value below the slash, when present, is the MIC in the presence of human serum.

The compounds of the invention can be prepared by the N-acylation of a 7-amino-3-halo-3-cephem-4-carboxylic acid or an ester thereof, for example the benzyl, p-methoxybenzyl, p-nitrobenzyl, diphenylmethyl, 2,2,2-trichloroethyl, trimethylsilyl or t-butyl ester, with an active derivative of a phenyl, thienyl or furyl substituted glycine of the formula



wherein R has the same meanings as defined in Formula I. In carrying out the acylation the amino group of the glycine is desirably protected as, for example, a salt such as the hydrochloride or with one of the commonly used amino-protecting groups, for example, the t-butyloxycarbonyl, benzyloxycarbonyl, p-nitrobenzyloxycarbonyl, trichloroethoxycarbonyl, or trityl group, or enamines formed with methyl acetoacetate and acetylacetone and like groups. Activated derivatives of the carboxyl group of the substituted glycine can be the acid halides such as the acid chlorides, the activated esters such as those formed with pentachlorophenol, the azide, or the mixed anhydride formed with the glycine and methyl chloroformate and isobutyl chloroformate. Also, the amino-protected glycine can be used directly in the acylation of the desired 3-halo nucleus ester by employing a condensing agent such as N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). For example, p-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate is reacted with N-(t-butyloxycarbonyl) D-phenyl-glycine in a dry inert solvent, for example tetrahydrofuran, to form p-nitrobenzyl 7-(D- α -phenylglycyl-

amido)-3-chloro-3-cephem-4-carboxylate. In general, any of the known amide coupling methods can be used in the acylation of the 7-amino-3-halo-3-cephem-4-carboxylic acids or esters. When the activated derivative is an acid halide the acylation is carried out in the presence of a hydrogen halide acceptor such as sodium bicarbonate, pyridine, sodium bisulfite, or any alkylene oxide such as propylene oxide. When a mixed anhydride is used in the acylation the mixed anhydride may be formed with the use of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). When an N-protected phenyl, thienyl or furylglycine is used, the acylation can be carried out in the presence of a condensing agent such as N,N'-dicyclohexylcarbodiimide.

Illustrative of the substituted glycines which can be used in the preparation of the compounds of the invention are D-phenylglycyl chloride hydrochloride, D-4-hydroxyphenylglycyl chloride hydrochloride, pentachlorophenyl D-phenyl-N-(t-butyloxycarbonyl)glycinate, pentachlorophenyl D-2-thienyl-N-(2,2,2-trichloroethoxycarbonyl)-glycinate, N-(t-butyloxycarbonyl)-D-phenylglycine, N-(1-carbomethoxy-2-propenyl)-D-phenylglycine, 3-thienylglycyl chloride hydrochloride, N-(t-butyloxycarbonyl)-2-thienylglycine or N-(t-butyloxycarbonyl)-2-furylglycine.

The acylation is usually carried out in an inert solvent for example in acetone, acetonitrile, dimethylformamide or methylene chloride and preferably at a temperature between about -20 and 20°C. For example, 7-(D-phenylglycyl-amido)-3-chloro-3-cephem-4-carboxylic acid is prepared by reacting D-phenylglycyl chloride hydrochloride with p-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate in acetonitrile in the presence of propylene oxide. The ester group can then be removed by hydrogenolysis.

Illustrative of the 7-amino-3-halo-3-cephem-4-carboxylic acids and esters which are starting materials in the preparation of compounds of formula I are 7-amino-3-chloro-3-cephem-4-carboxylic acid, 7-amino-3-fluoro-3-cephem-4-carboxylic acid, p-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate, diphenylmethyl 7-amino-3-bromo-3-cephem-4-carboxylate, diphenylmethyl 7-amino-3-chloro-3-cephem-4-carboxylate and p-methoxybenzyl 7-amino-3-fluoro-3-cephem-4-carboxylate.

The 7-amino-3-halo-3-cephem-4-carboxylic acids and esters can be prepared in the following manner. A 7-acylamido cephalosporanic acid or an ester thereof is converted to a 7-acylamido-3-exomethylenecepham-4-carboxylic acid or ester. An ester of the 3-exomethylenecepham compound is then oxidized with ozone to form an intermediate ozonide involving the 3-exomethylene grouping, which ozonide on decomposition provides the 7-acylamido-3-hydroxy-3-cephem-4-carboxylic acid ester. The 3-hydroxy-3-cephem ester is then fluorinated, chlorinated, or brominated to provide the corresponding 3-halo-3-cephem. The 7-acyl group of the 3-halo cephem can then be removed by the well-known side chain cleavage reaction by employing phosphorus pentachloride in pyridine to obtain the imino chloride derivative of the side chain, the imino chloride then being reacted with an alcohol such as methanol to provide the imino ether, which can then be readily hydrolyzed to provide the 7-amino-3-halo-3-cephem-4-carboxylic acid ester.

For example, a 7-acylamidocephalosporanic acid such as 7-phenoxyacetamidocephalosporanic acid is reacted with a sulfur-containing nucleophile according to known procedures to effect a nucleophilic displacement of the acetoxymethyl group of the cephalosporanic acid and provide a 7-acylamido-3-thio-substituted-methyl-3-cephem-4-carboxylic acid. The 3-thio-substituted-cephem acid is then reduced with zinc/formic acid in the presence of dimethylformamide (DMF) or with Raney nickel in the presence of hydrogen to yield a 7-acylamido-3-exomethylenecepham-4-carboxylic acid. For example, 7-phenoxyacetamido-3-acetoxymethyl-3-cephem-4-carboxylic acid is reacted with potassium ethylxanthate to provide 7-phenoxyacetamido-3-ethoxythionocarbonylthiomethyl-3-cephem-4-carboxylic acid. Reduction of the latter compound with zinc and formic acid in the presence of DMF provides 7-phenoxyacetamido-3-exomethylenecepham-4-carboxylic acid. Likewise, any of a wide variety of 7-acylamidocephalosporanic acids can be reacted with a variety of sulfur-containing nucleophiles to provide the 3-thio-substituted-methyl-3-cephem compounds. For example the 7-acylamido group can be a heterocyclic containing chain for example 2-thienylacetamido, or 2-furylacetamido, or it can be an alkanoyl side chain as exemplified by acetamido or a wide variety of other side chains. Also, the sulfur-containing nucleophile can be any of a wide variety of compounds such as thiourea and substituted thioureas which react with the cephalosporanic acids to provide isothiuronium salts, the thiobenzoates,

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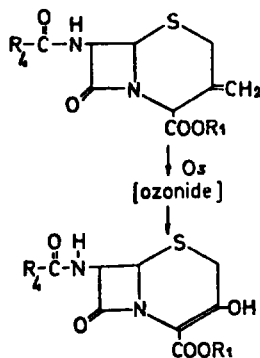
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mercaptopyridine N-oxide, 1-methyl-tetrazol-5-thiol, 5-methyl-1,3,4-thiadiazol-2-thiol, and other sulfur-containing nucleophiles. Following the reductive displacement reaction described above the 3-exomethylenecephem-4-carboxylic acid thus prepared is then esterified for example with p-nitrobenzyl bromide, p-methoxybenzyl bromide, diphenyldiazomethane, or 2,2,2-trichloroethyl chloroformate or other ester forming compounds, and the 3-exomethylenecephem ester is reacted with ozone to provide the 3-hydroxy-3-cephem ester. The ozonolysis reaction of a 3-exomethylenecephem ester is carried out in an inert solvent at a temperature between about -80 and -0°C . and preferably between -80 and -50°C . to form an intermediate ozonide. The ozonide is then decomposed, *in situ* in the cold to provide the corresponding 3-hydroxy-3-cephem ester as illustrated in the following reaction scheme.



In the above formula R_4 is H or the residue of the acylamido group as described above for example R_4 can be benzyl, phoxymethyl, methyl, 2-thienylmethyl or 2-furylmethyl, and R_1 is as defined in the formula 1.

The ozonolysis of a 3-exomethylenecephem ester is carried out by bubbling ozone into a solution of the ester in an inert solvent until ozonide formation is complete. Inert solvents which can be used in the ozonolysis method are those solvents in which the 3-exomethylenecephem esters are at least partially soluble and which are unreactive with ozone under the described conditions. Commonly used solvents such as methanol, ethanol, ethyl acetate, methyl acetate, iso-amyl acetate, and methylene chloride are satisfactory solvents.

Ozone gas is prepared by means of an ozone generator of the type commonly used in synthetic and analytical chemical work. Such generators produce ozone by the action of an electric discharge on oxygen. One such ozone generator is that manufactured by the Wellsback Corporation. The ozone is generated in a stream of oxygen which is then passed directly into the reaction vessel. The percentage of ozone contained in the oxygen stream can be varied as desired, for example, by varying the rate of flow of oxygen through the ozonizer as well as by varying the intensity of the electric discharge.

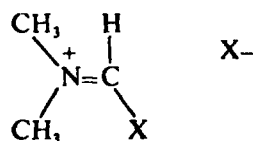
The concentration of the 3-exomethylenecephem ester starting material in the inert solvent is not critical and it is preferred to use a solvent volume sufficient to form a complete solution.

When ozonide formation is complete, any excess ozone present in the reaction mixture is purged from the mixture by bubbling nitrogen, oxygen or an inert gas such as argon through the mixture. Following the removal of any excess ozone, the ozonide is decomposed by adding to the reaction mixture a reducing agent selected from the group consisting of sodium bisulfite, sulfur dioxide, and trimethylphosphite to provide the 3-hydroxy-3-cephem-4-carboxylic acid ester. The decomposition is carried out by adding an excess of the decomposition reagent and then stirring the reaction mixture until the reaction mixture is negative to the potassium iodide-starch test. A preferred reagent for decomposing the intermediate ozonide is gaseous sulfur dioxide.

The 3-hydroxy-3-cephem esters are recovered from the reaction mixture by evaporating off the volatile solvents from the mixture to obtain the reaction product mixture as a residue and thereafter recrystallizing the reaction product from the residue.

The 7-acylamido-3-hydroxy-3-cephem-4-carboxylic acid esters thus prepared are then halogenated to provide the 7-acylamido-3-halo-3-cephem-4-carboxylic acid ester.

The compounds represented by the formula I wherein X is chloro or bromo can be prepared by reacting a 7-acylamido-3-hydroxy-3-cephem ester or a 3-hydroxy-3-cephem nucleus ester in dimethylformamide (DMF) with a reactive chloro or bromo compound which forms with DMF the chloro or bromo dimethyliminium chloride or bromide as represented by the formula

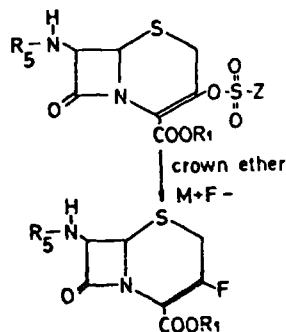


wherein X and X⁻ represent chloro or bromo and chloride or bromide respectively. The reactive halo iminium halide of the above formula is formed *in situ* and is a highly reactive chlorinating or brominating intermediate. Chloro and bromo compounds which form the above iminium halide include the commonly used chlorinating agents such as phosgene (carbonyl chloride), oxalyl chloride, thionyl chloride, and the phosphorus chlorides, for example, phosphorus trichloride and phosphorus oxychloride (phosphoryl chloride). Brominating reagents which can be employed in the present invention include carbonyl dibromide, oxalyl bromide, thionyl bromide (sulfurous oxybromide), and the phosphorus bromides, phosphorus oxybromide, and phosphorus tribromide. Phosphorus pentachloride can be employed in the preparation of the 3-chloro-3-cephem compounds of the invention, however this reagent concurrently reacts with the 7-acylamido side chain of the starting material to form the imino chloride, the reactive intermediate in the well known cephalosporin side chain cleavage reaction. Accordingly, it is preferable to use one of the other named chlorinating agents.

The chlorination and bromination can be carried out by adding the halogenating reagent to a solution of the 3-hydroxy-3-cephem ester in dry DMF at a temperature of about 5° to 15°C. and allowing the reaction mixture to stand at room temperature for between 4 and 8 hours or longer. The reaction is initially exothermic and accordingly the reaction vessel is initially cooled in an ice-water bath and then maintained at about 25°C. during the remainder of the reaction. The DMF is preferably dried prior to use over a molecular sieve. Although the reaction can be carried out in DMF as the solvent, a cosolvent may also be employed along with the DMF. For example, a cosolvent such as tetrahydrofuran, dioxan, methylene chloride, dimethylacetamide or dimethylsulfoxide can be used along with DMF. Other solvents capable of forming the iminium halide can also be used in conjunction with or instead of DMF.

The 3-chloro or 3-bromo-3-cephem esters are recovered from the reaction product mixture by pouring the mixture into a water-ethyl acetate mixture and separating the organic phase containing the product. The organic phase is washed, dried and is then evaporated to provide the 3-halo-3-cephem ester as an amorphous residue. The product is obtained crystalline in many instance by trituration of the residue with ether or with n-hexane.

The compounds represented by the formula I wherein X is fluoro and R₁ is other than hydrogen can be prepared from the alkyl sulfonate or arylsulfonate esters of the 3-hydroxy-3-cephem esters by the following generalized reaction scheme:



wherein M is potassium, sodium or silver, R₁ is an acyl group and Z is C₁₋₆ alkyl or an optionally substituted phenyl group.

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C₁₋₆ alkyl or

Crown ethers are described in *Tetrahedron Letters*, 18, 1793—6 (1972).

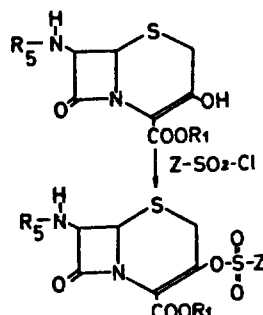
The reaction can be carried out in an inert solvent, specifically a nitrile or a nitroalkane, preferably acetonitrile, propionitrile, nitromethane, or nitroethane, under substantially anhydrous conditions at a temperature of from about -20°C. to about room temperature and preferably at about room temperature. The 3-sulfonate ester cephalosporin present in the selected solvent is mixed with a maximum of one equivalent of the fluoride source based on the sulfonate ester and at least an equivalent amount of crown ether based upon the fluoride which is present. The reaction is completed generally within a period of from 30 minutes to 3 hours, with time generally being dependent upon the temperature of reaction. Typical crown ethers include 18-crown-6-ether as well as corresponding substituted 18-crown-6-ether, such as dicyclohexyl-18-crown-6-ether. Preferably, the fluoride source is an inorganic fluoride salt, M⁺F⁻ hereinabove being, for example, sodium fluoride, or silver fluoride.

The product which is obtained from the reaction will correspond structurally in every respect to the sulfonate ester starting material with the exception that the group —O—SO₂—Z will have been displaced by a fluorine atom.

It has been discovered that the initial 3-sulfonate ester cephalosporin reactant can be either a Δ³-cephalosporin, a Δ² cephalosporin, or a mixture of the two. The active reactant is the Δ³-cephalosporin. However, it has been discovered that, under the conditions of reaction, any Δ²-cephalosporin which may be present is isomerized to the Δ³-cephalosporin, and thus the active reactant is formed *in situ* if not present initially in the reaction mixture.

Isolation of the resulting product can be accomplished employing generally recognized techniques. Preferably, the product is isolated by means of preparative thin layer chromatography employing customary procedures.

The preparation of the alkylsulfonate and arylsulfonate esters of the 3-hydroxy-3-cephem esters is illustrated in the following generalized reaction scheme.



In the above formula, the terms R₅ and R₁ have the same meanings as previously defined and Z is C₁—C₆ alkyl, such as methyl, ethyl, propyl, n-butyl or n-hexyl, or Z is a phenyl group or a substituted phenyl group such as methylphenyl, nitrophenyl, or halophenyl. Representatives of the sulfonyl halides which can be employed in the preparation of these sulfonate esters are methanesulfonyl chloride, toluenesulfonyl chloride, p-fluorobenzene sulfonyl chloride, ethanesulfonyl chloride, or butanesulfonyl chloride.

The reaction can be carried out in an inert solvent at a temperature between about -5 and 35°C., and preferably between about 15 and 25°C. Inert solvents which can be employed in the sulfonation reaction are generally aprotic solvents, and those solvents which are unreactive with the sulfonyl halide. Solvents which can be employed include the ether solvents such as tetrahydrofuran and dioxan. A preferred solvent for the sulfonation reaction is dimethylacetamide. The reaction is carried out in the presence of a hydrogen halide acceptor, and the commonly employed tertiary amines such as pyridine and triethylamine can be used, however, the preferred hydrogen halide acceptor in this process is propylene oxide. Other alkylene oxides can be employed, however, propylene oxide is much preferred. The tertiary amine hydrogen halide acceptors are less desirable than the alkylene oxides in that the double bond in the delta 3 position of the cephem ring is susceptible to isomerization to the delta 2 position in the presence of these amines. However, should isomerization to the delta 2 isomer occur during sulfonate ester formation, the double bond can be isomerized back to the delta 3 position in the following manner. The delta 2 isomer of the sulfonate ester is first oxidized with a peracid, such as m-chloroperbenzoic acid or peracetic acid to the sulfoxide.

During sulfoxide formation the double bond shifts from the delta 2 to the delta 3 position and thereafter the sulfoxide is reduced, for example, with phosphorus trichloride, according to the well known method to provide the 3-cephem sulfonate ester.

A preferred sulfonate ester for the conversion of the 3-hydroxy-3-cephem-4-carboxylic acid esters to the corresponding 3-fluoro-3-cephem compounds is the methylsulfonate ester (mesylate). Another preferred ester is that formed with p-toluenesulfonyl chloride (tosylate ester). A preferred C₄ carboxylic acid protecting ester group in the above described process is the p-nitrobenzyl ester.

Alternatively, the fluorination of a 3-hydroxy-3-cephem ester is carried out by reacting a 7-acylamido-3-hydroxy-3-cephem ester in an inert solvent with the fluorinated tertiary amine, N-(2-chloro-1,1,2-trifluoroethyl)diethylamine. The fluorinated tertiary amine can be prepared by reacting diethylamine with chlorotrifluoroethylene as described in *J. Org. Chem.* 29, 2187 (1964); *Tetrahedron Lett.* 23, 1065 (1962) and *Tetrahedron Lett.* 26, 1249 (1962). An equivalent amount of the fluorinated tertiary amine is employed in an inert solvent, for example methylene chloride, chloroform, tetrahydrofuran, or any unreactive solvent in which the starting material and fluorinating reagent are substantially soluble. The reaction mixture containing the 3-hydroxy ester and the fluorinating reagent is heated in the inert solvent at a temperature between about 30 and about 60°C. for about 1 hour. The fluoro cephem ester is recovered by following recovery procedures analogous to those described for the recovery of the 3-chloro and 3-bromo compounds.

Following the preparation of the 7-acylamido-3-halo-3-cephem-4-carboxylic acid esters as described above the respective 7-amino-3-halo-3-cephem-4-carboxylic acid esters are obtained by carrying out the well-known N-deacylation of the 7-acylamido side chain. For example the 7-acylamido-3-halo cephalosporin ester is reacted with phosphorus pentachloride in methylene chloride in the presence of pyridine to form the intermediate imino chloride. The imino chloride is then reacted with an alcoholic solvent such as methanol or isobutanol to provide the corresponding imino ether. The imino ether is then hydrolyzed to provide the 7-amino-3-halo-3-cephem-4-carboxylic acid ester as a hydrochloride salt.

As previously mentioned the 7-amino-3-halo starting material can be acylated either as the free acid or as an ester thereof. The acylation of the 7-amino-3-halo nucleus is carried out as previously described.

In a specific embodiment of the preparation of starting materials and the compounds of the invention, 7-phenoxyacetamidocephalosporanic acid is reacted with thio-urea to provide the isothiuronium salt by the displacement of the acetoxy group in the 3-position of the cephalosporanic acid dihydrothiazine ring. The isothiuronium salt is then reacted with zinc and excess 90% formic acid in the presence of dimethylformamide at a temperature of about 25° to provide the 7-phenoxyacetamido-3-exomethylenecepham-4-carboxylic acid. The 3-exomethylenecepham carboxylic acid is esterified with p-nitrobenzyl bromide in the presence of a hydrogen halide acceptor to provide the 3-exomethylenecepham-4-carboxylic acid p-nitrobenzyl ester. The 3-exomethylenecepham ester is then ozonized in methylene chloride at a temperature of about -70° and the ozonolysis mixture is treated with sulfur dioxide to decompose the intermediate ozonide to yield p-nitrobenzyl 7-phenoxyacetamido-3-hydroxy-3-cephem-4-carboxylate. The 3-hydroxy ester is then reacted with phosphorus trichloride in dry DMF to provide p-nitrobenzyl 7-phenoxyacetamido-3-chloro-3-cephem-4-carboxylate. The 3-chloro ester is then reacted in methylene chloride with phosphorus pentachloride in the presence of pyridine to provide, in situ, the imino chloride intermediate, which is then reacted with methanol to provide the corresponding imino ether intermediate. Upon addition of water to the reaction mixture the imino ether decomposes to provide the p-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate. The 7-amino-3-chloro-3-cephem-4-carboxylic acid ester can then be acylated, as previously described herein, with D-phenylglycyl chloride hydrochloride or with an amino-protected D-phenylglycine derivative to provide the 7-(D-phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid p-nitrobenzyl ester or an N-protected derivative thereof. After removal of the 7-amino protecting group and the C₄ carboxyl ester group, 7-(D-phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid is obtained. It will be recognized from the foregoing description of the preparation of the starting materials that a wide variety of known 7-acylamidocephalosporanic acids can be employed in the preparation of the 7-amino-3-halo-3-cephem-4-carboxylic acids described herein.

A solution of 2.5 g. of p-methoxybenzyl 7-phenoxy-acetamido-3-methylene-cepham-4-carboxylate in 350 ml. of ethyl acetate was cooled in an acetone-dry ice

bath. Ozone was bubbled through the cold solution for 8 minutes and then oxygen was passed through the ozonized reaction mixture to expel excess ozone. The intermediate ozonide was decomposed by adding to the reaction mixture 25 g. of sodium bisulfite with stirring at a temperature of about 0°C. The reaction solution was decanted and was washed successively with water, 5% hydrochloric acid and a saturated solution of sodium chloride. The washed mixture was dried and evaporated to yield the reaction product, p-methoxybenzyl 7-phenoxyacetamido-3-hydroxy-3-cephem-4-carboxylate as an amorphous solid.

10 N.M.R. (CDCl₃): signals at
 6.73 (s, 2H, C₂H₂),
 6.23 (s, 3H, p-methoxy),
 5.53 (s, 2H, side-chain CH₂),
 5.03 (d, 1H, C₆H),
 15 4.87 (s, 2H, ester CH₂),
 4.47 (q, 1H, C₇H),
 3.40—2.50 (m, 9H, aromatic H),
 2.33 (d, 1H, amide NH), and
 1.53 (broad s, 1H, 3 OH) tau.

20 Example 4.

p-Nitrobenzyl 7-[2-(2-thienyl)acetamido]-3-hydroxy-3-cephem-4-carboxylate

To a solution of 1.55 g. of p-nitrobenzyl 7-amino-3-hydroxy-3-cephem-4-carboxylate hydrochloride in 30 ml. of acetone containing 364 mg. (0.5 ml., 3.6 mmole) of triethylamine was added 962 mg. of urea. With stirring at room temperature, a solution of 730 mg. (4.4 mmole) of 2-thiophene acetyl chloride in 20 ml. of acetone was added dropwise to the mixture. After 2.5 hours the reaction mixture was filtered and evaporated. The residue was dissolved in ethyl acetate and the solution was washed successively with water, a 5% solution of sodium bicarbonate, 5% hydrochloric acid, and a saturated solution of sodium chloride. The washed solution was dried and then was concentrated by evaporation in vacuo to yield 1.2 g. of the reaction product as a crystalline residue. The product was recrystallized from ethyl acetate to yield pure p-nitrobenzyl 7-[2-(2-thienyl)acetamido]-3-hydroxy-3-cephem-4-carboxylate having the following spectral properties.

35 I.R. (Nujol Mull): absorption peaks at 3.0 (amide NH), 5.68 (β-lactam carbonyl), and 6.1 (amide, and ester hydrogen bonded to 3 OH) microns.
 N.M.R. (CDCl₃/DMSO d₆): signals at 6.54 (2d, 2H, C₂H₂), 6.16 (s, 2H, side-chain CH₂), 4.90 (d, 1H, C₆H), 4.60 (d, 2H, ester CH₂), 4.43 (q, 1H, C₇H), 3.1—1.6 (m, 7H, aromatic H) and 1.30 (d, 1H, amide NH) tau.

40 Example 5.

p-Nitrobenzyl 7-acetamido-3-hydroxy-3-cephem-4-carboxylate

A solution of 10 mmole of p-nitrobenzyl 7-amino-3-hydroxy-3-cephem-4-carboxylate hydrochloride in a mixture of 325 ml. of acetone and 125 ml. of water was cooled in an ice-water bath. With stirring a stream of ketene gas was bubbled through the solution for 30 minutes. Thereafter the reaction mixture was evaporated to remove acetone and the aqueous residue was slurried with ethyl acetate. The ethyl acetate layer was separated and was washed with 5% hydrochloric acid and a saturated solution of sodium chloride. The washed extract was dried and evaporated in vacuo to yield the reaction product as a crystalline residue. The residue was triturated with diethyl ether and vacuum dried to yield 3.55 g. of p-nitrobenzyl 7-acetamido-3-hydroxy-3-cephem-4-carboxylate melting at about 146—152°C. with decomposition.

Elemental analysis for: C₁₆H₁₃N₃O₇S:

55 Theory: C, 48.85; H, 3.84; N, 10.68
 Found: C, 48.97; H, 3.96; N, 10.42.

I.R. (CHCl₃): absorption bands at 2.9 and 3.0 (amide NH and OH), 5.63 (β-lactam carbonyl) and 5.95 (broad, amide, and ester carbonyl hydrogen bonded to 3 OH) microns.

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N.M.R. (CDCl₃): signals at 7.90 (s, 3H, 7-acetamido CH₃), 6.55 (s, 2H, C₂H₂),
4.92 (d, 1H, C₆H), 4.63 (m, 2H, ester CH₂), 4.30 (q, 1H, C₇H), 2.81
(d, 1H, amide NH), 2.5—1.8 (m, 4H, aromatic H), and 2.8 (s, 1H, C₃ OH) tau.
Electrometric titration (66% aqueous DMF)
pKa 5.9

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Example 6.

Diphenylmethyl 7-[2-(2-thienyl)acetamidol-3-chloro-3-cephem-4-carboxylate
a) To a solution of 34 g. (100 mmole) of 7-[2-(2-thienyl)acetamidol-3-
methyleneccepham-4-carboxylic acid in 500 ml. of methylene chloride was added
21.4 g. (110 mmole) of diphenyl diazomethane and the resulting mixture was
stirred for 2 hours at room temperature. The solvent was evaporated under
reduced pressure and the residue was dissolved in ethyl acetate. The ethyl acetate
solution was washed with a 5% solution of sodium bicarbonate, then with water
and dried over magnesium sulfate. The dried solution was concentrated to a small
volume. On standing 40 g. of diphenylmethyl 7-[2-(2-thienyl)acetamidol-3-
methyleneccepham-4-carboxylate melting at about 132—133°C. precipitated as a
crystalline solid.

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IR (chloroform): absorption peaks at 2.9 (amide N—H), 5.65, 5.75 and 5.93
(β-lactam, ester and amide carbonyls respectively) and 6.62 (amide II)
microns

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NMR (CDCl₃): signals at 6.72 (ABq, 2H, C₂—H₂), 6.21 (s, 2H, α-CH₂), 4.83—4.65
(m, 4H, C₄—H, C₆—H and C₇—CH₂), 4.39 (q, 1H, C₇—H), 3.4—2.65 (m, 15H,
C₇—NH, ester CH and aromatic H) tau.

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b) To the solution of 8.1 g (16 mmole) of the above ester in 80 ml. of
methylene chloride were added 1.57 g. (1.6 ml, 19.6 mmole) of dry pyridine and 3.8
g. (18.1 mmole) of phosphorous pentachloride. The reaction mixture was stirred
for 2 hours at room temperature and was thereafter cooled in an ice-water bath.
The cold mixture was treated with 8 ml. of isobutanol with stirring. Stirring was
continued for 2 hours during which time 3 g. of diphenylmethyl 7-amino-3-
methyleneccepham-4-carboxylate hydrochloride formed as a crystalline pre-
cipitate. The product was filtered and washed with methylene chloride and
vacuum dried.

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Elemental analysis (percent) for C₂₁H₂₁N₂O₂SCl:

Theory: C, 60.50; H, 5.08; N, 6.72; Cl, 8.50;

Found: C, 60.70; H, 5.02; N, 6.71; Cl, 8.80.

NMR (DMSO d₆): signals at 6.45 (ABq, 2H, C₂—H₂), 5.00 (d, 1H, C₆—H), 4.68
(d, 1H, C₇—H), 4.60 (s, 2H, 3-CH₂), 4.44 (s, 1H, C₄—H), 3.10 (s, 1H, ester CH),
and 2.61 (s, 10H, aromatic H) tau.

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(carbonyl),
side-chain
1), 3.1—1.6

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c) The 7-amino-3-exomethyleneccepham ester hydrochloride salt product, 2.1 g.
(5 mmole) was dissolved in 200 ml. of methanol and the solution was cooled in an
acetone-dry ice bath. Ozone was bubbled into the cold solution for 7 minutes to
form the intermediate ozonide. The ozonide was decomposed by passing a stream
of sulfur dioxide gas through the reaction mixture for 2 minutes. Thereafter the
reaction mixture was evaporated and the residue was triturated with diethyl ether
to yield 1.6 g. of diphenylmethyl 7-amino-3-hydroxy-3-cephem-4-carboxylate
hydrochloride as a crystalline solid.

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NMR (CDCl₃): signals at 6.4 (ABq, 2H, C₂—H₂), 5.0—4.5 (m, 2H, C₆—H and
C₇—H), 3.2—2.4 (m, 11H, ester CH and aromatic H) tau.

IR (chloroform): carbonyl absorption peaks at 5.57 and 5.70 (β-lactam and
ester carbonyl respectively) microns.

UV (pH7 buffer): λ max 275 mμ, ε=7550.

Electrometric titration (60% aq. DMF): titratable groups at 4.5 and 6.5.

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d) To a solution of 840 mg. of diphenylmethyl 7-amino-3-hydroxy-3-cephem-
4-carboxylate in 10 ml. of water and 10 ml. of acetone was added one gram of
sodium bisulfite. The mixture was stirred and 800 mg. of thiophene-2-acetyl
chloride in 10 ml. of acetone were added dropwise. The mixture was stirred for 4.5
hours at room temperature and was then evaporated under reduced pressure. The
residue was dissolved in a mixture of ethyl acetate and an aqueous 5% solution of

(OH), 5.63
yl hydrogen

sodium bicarbonate. The ethyl acetate layer was separated, washed with water and dried. The dried solution was evaporated and the residue triturated with ether to yield 500 mg. of diphenylmethyl 7-[2-(2-thienyl)acetamidol]-3-hydroxy-3-cephem-4-carboxylate.

- 5 NMR (CDCl_3): signals at 6.79 (s, 2H, $\text{C}_2\text{--H}_2$), 6.16 (s, 2H, $\alpha\text{--CH}_2$), 5.0 (d, 1H, $\text{C}_6\text{--H}$), 4.32 (q, 1H, $\text{C}_7\text{--H}$), 3.05—2.46 (m, 15H, $\text{C}_7\text{--NH}$, ester CH and aromatic H) tau.
 10 I.R. (chloroform): absorption peaks at 2.9 (amide NH), 5.6, 5.73 and 5.95 (β -lactam, ester and amide carbonyls respectively) and 6.65 (amide II) microns.

- 15 e) To a solution of 4.2 g. of diphenylmethyl 7-[2-(2-thienyl)acetamidol]-3-hydroxy-3-cephem-4-carboxylate in 44 ml. of dry dimethylformamide was added 865 mg. of phosphorus trichloride. The mixture was stirred for 1.5 hours at room temperature and was poured into an ethyl acetate 5% aqueous hydrochloric acid mixture. The ethyl acetate layer was evaporated, was washed with 5% hydrochloric acid, water and was dried. The dried solution was concentrated in vacuo and the product crystallized. The 3-chloro ester was filtered, washed with cold ethyl acetate and dried to yield 2.2 g.

- 20 Elemental analysis (percent) for $\text{C}_{26}\text{H}_{21}\text{N}_3\text{O}_4\text{S}_2\text{Cl}$:
 Theory: C, 59.48; H, 4.03; N, 5.34; Cl, 6.75
 Found: C, 59.77; H, 4.25; N, 5.40; Cl, 6.91.
 NMR (CDCl_3): signals at 6.49 (ABq, 2H, $\text{C}_2\text{--H}_2$), 6.22 (s, 2H, $\alpha\text{--CH}_2$), 5.08 (d, 1H, $\text{C}_6\text{--H}$), 4.19 (q, 1H, $\text{C}_7\text{--H}$), 3.13—2.5 (m, 15H, $\text{C}_7\text{--NH}$, ester CH, and aromatic H) tau.
 25 I.R. (CHCl_3): absorption peaks at 2.9 (amide NH), 5.55, 5.72 and 5.90 (β -lactam, ester and amide carbonyls) and 6.60 (amide II) microns.
 U.V. (dioxan): λ max 275 $\text{m}\mu$, $\epsilon=8700$.

Example 7.

- 30 p-Nitrobenzyl 7 - [2-(2-thienyl)acetamidol]-3-chloro-3-cephem-4-carboxylate. (via thionyl chloride)
 To a solution of 1.9 g. (4 mmole) of p-nitrobenzyl 7-[2-(2-thienyl)acetamidol]-3-hydroxy-3-cephem-4-carboxylate in 10 ml. of DMF (dried over a molecular sieve) was added 950 mg. (0.58 ml., 8 mmole) of freshly distilled thionyl chloride. The mixture was stirred at room temperature for 6.5 hours and was then poured
 35 into 100 ml. of ethyl acetate. The mixture was extracted three times with 30 ml. portions of 5% hydrochloric acid and with a saturated solution of sodium chloride. The washed ethyl acetate solution was filtered and evaporated to dryness in vacuo. The residue was triturated with ether to yield 1.2 g. of p-nitrobenzyl 7-[2-(2-thienyl)acetamidol]-3-chloro-3-cephem-4-carboxylate as a brown crystalline solid
 40 melting at 164—166°C.

- Elemental analysis (percent) for $\text{C}_{20}\text{H}_{16}\text{N}_3\text{O}_6\text{S}_2\text{Cl}$:
 Theory: C, 48.63; H, 3.27; N, 8.51; Cl, 7.18
 Found: C, 48.47; H, 3.29; N, 8.78; Cl, 6.96
 IR (Chloroform) showed absorption bands at 2.9 (amide NH), 5.59 (β -lactam carbonyl), 5.75 (ester carbonyl) and 5.92 microns (amide carbonyl).
 45 UV absorption spectrum (acetonitrile) showed maxima at
 λ max 235 $\text{m}\mu$, $\epsilon = 12,100$
 and λ max 268 $\text{m}\mu$, $\epsilon = 15,800$.
 The mass spectrum of the product showed a molecular ion of 493 m/e.
 50 NMR (CDCl_3) showed signals at 6.39 (ABq, 2H, $\text{C}_2\text{--H}_2$), 6.17 (s, 2H, $\alpha\text{--CH}_2$), 4.99 (d, 1H, $\text{C}_6\text{--H}$), 4.64 (s, 2H, ester CH_2), 4.19 (q, 1H, $\text{C}_7\text{--H}$), 3.45 (d, 1H, $\text{C}_7\text{--NH}$), 3.1—1.67 (m, 7H, aromatic H) tau.

Example 8.

- 55 p-Nitrobenzyl 7 - [2-(2-Thienyl)acetamidol]-3-chloro-3-cephem-4-carboxylate (via phosphorus trichloride)
 To a cooled solution of 439 mg. (0.93 mmole) of p-nitrobenzyl 7-[2-(2-thienyl)acetamidol]-3-hydroxy-3-cephem-4-carboxylate in 4.4 ml. of DMF was slowly added 85 mg. (0.05 ml., 0.63 mmole) of phosphorus trichloride. The reaction mixture was allowed to stand for 4 hours at room temperature and thereafter

with water
with ether
hydro. 3-

l, 5.0 (d,
ester CH

and 5.95
amide II)

amidol-3-
was added
rs at room
hloric acid
5% hydro-
d in vacuo
l with cold

(CH₂), 5.08
C₇-NH,

and 5.90
ns.

oxylate. (via
acetamidol-
a molecular
yl chloride.
hen poured
with 30 ml.
m chloride.
ss in vacuo.
zyl 7-[2-(2-
talline solid

59 (3-lactam
nyl).

m/e.
2H, α CH₂),
C₇-H), 3.45

boxylate (via
2-(2-thienyl)-
was slowly
The reaction
nd thereafter

the reaction product mixture was worked-up by following the recovery procedures described in Example 6 to provide 374 mg. of p-nitrobenzyl 7-[2-(2-thienyl)-acetamidol-3-chloro-3-cephem-4-carboxylate. The NMR spectrum of the product was consistent with the expected product and with that of the product of Example 7.

Example 9.

7-Phenoxyacetamido-3-chloro-3-cephem-4-carboxylic acid

Following the chlorination procedure of Example 6 p-nitrobenzyl 7-phenoxyacetamido-3-chloro-3-cephem-4-carboxylate was prepared with phosphorus trichloride. The p-nitro-benzyl ester group was removed by hydrogenolysis with hydrogen and 5% Pd/C to provide the 3-chlorocephalosporanic acid antibiotic compound.

Example 10.

p-Nitrobenzyl 7-[2-(2-thienyl)acetamidol-3-chloro-3-cephem-4-carboxylate (via Oxalyl chloride)

To a solution of 439 mg. (0.93 mmole) of p-nitro-benzyl 7-[2-(2-thienyl)-acetamidol-3-hydroxy-3-cephem-4-carboxylate in 4.4 ml. of DMF cooled in an ice bath was added dropwise 118 mg. (0.07 ml., 0.93 mmole) of oxalyl chloride. The reaction mixture was allowed to stand for 4 hours at room temperature and was then poured into a mixture of aqueous 5% hydrochloric acid and ethyl acetate. The organic layer was separated and was washed sequentially with 5% hydrochloric acid, water and a saturated solution of sodium chloride. The washed layer was dried and evaporated to dryness to yield the reaction product, p-nitrobenzyl 7-[2-(2-thienyl)acetamidol-3-chloro-3-cephem-4-carboxylate as an amorphous solid. The product was obtained crystalline by triturating the amorphous residue with ether. Yield 360 mg. The infrared spectrum and NMR spectrum of the crystalline product were consistent with the spectra of authentic material.

Example 11.

7-[2-(2-Thienyl)acetamidol-3-bromo-3-cephem-4-carboxylic acid

To a solution of 19 g (40 mmole) of p-nitrobenzyl 7-[2-(2-thienyl)-acetamidol-3-hydroxy-3-cephem-4-carboxylate in 300 ml of dry DMF was added 15 g (56 mmole) of phosphorus tribromide and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into a mixture of ethyl acetate and water and the organic phase was separated and washed repeatedly with water and dried over magnesium sulfate. The dried organic phase was evaporated in vacuo to dryness. The crude reaction product residue weighing about 9 g. was purified by chromatography over 500 g of silica gel using ethyl acetate-hexane (55:45 v:v) as eluent. The eluate was evaporated to dryness under reduced pressure and the product, p-nitrobenzyl 7-[2-(2-thienyl)-acetamidol-3-bromo-3-cephem-4-carboxylate was obtained crystalline by triturating the dry residue with diethyl ether.

U.V. (ethanol) λ max. 270 mμ (ε=13,300)

and λ max. 243 mμ (ε=12,700)

Elemental analysis calculated for C₂₀H₁₆BrN₃O₆S₂:

Theory: C, 44.61; H, 3.00; N, 7.81; Br, 14.84.

Found: C, 44.78; H, 3.03; N, 7.65; Br, 14.91.

Nuclear magnetic resonance spectrum (DMSO-d₆) showed signals at 6.21 (s, 2H α-CH₂), 5.98 (ABq, 2H, C₂-H₂), 4.72(d, 1HC₆-H), 4.51 (s, 2H, ester-CH₂), 4.20(q, 1H, C₇-H), 3.04-1.74 (m, 7H: aromatic H) and 0.66 (d, 1H, C₇-CH) tau.

The above 3-bromo ester was de-esterified in the following manner. The ester, 545 mg (1.0 mmole) was hydrogenated at room temperature in ethanol in the presence of pre-reduced 5 percent palladium-on-carbon catalyst. The catalyst was filtered and the filtrate evaporated under reduced pressure to dryness. The residual product was triturated with diethyl ether to yield 180 mg (44 percent) of crystalline product, 7-[2-(2-thienyl)acetamidol-3-bromo-3-cephem-4-carboxylic acid.

Electrometric titration (66 percent aqueous DMF) showed a pKa of 4.4 and an apparent molecular weight of 393.

The calculated molecular weight = 403.

Elemental analysis calculated for $C_{13}H_{11}BrN_2O_4S_2$, $\frac{1}{2}$ diethyl etherate:

Theory: C, 40.91; H, 3.66; N, 6.36; Br, 18.15.

Found: C, 41.29; H, 3.20; N, 6.29; Br, 18.50.

- 5 Nuclear magnetic resonance spectrum ($CDCl_3$) showed signals at 8.8 (t, diethyl ether- CH_2), 6.68—5.86 (m, C_5-H , $\alpha-CH_2$, and diethyl ether- CH_2), 4.90 (d, 1H, C_6-H), 3.0—2.63 (m, 3H, aromatic-H), and 1.9 (d, 1H, amide NH) tau.

Example 12.

- 10 7-[2-(2-Thienyl)acetamidol]-3-fluoro-3-cephem-4-carboxylic acid

To a solution of 325 mg. (0.7 mmole) of p-nitrobenzyl 7-[2-(2-thienyl)-acetamidol]-3-hydroxy-3-cephem-4-carboxylate in 10 ml. of methylene chloride was slowly added an equivalent amount of N-(2-chloro-1,1,2-trifluoroethyl) diethylamine. The reaction mixture was heated for 30 minutes under gentle reflux and was then evaporated to dryness *in vacuo*. The residue was dissolved in a mixture of ethyl acetate-water and the organic layer was separated. The organic layer was washed with 5% hydrochloric acid, water and brine and was then dried. The dried reaction product solution was concentrated to a small volume and n-hexane was added to precipitate, p-nitrobenzyl (7-[2-(2-thienyl)-acetamidol]-3-fluoro-3-cephem-4-carboxylate.

The ester group was removed by catalytic hydrogenolysis to yield the 3-fluoro cephalosporin free acid antibiotic.

Example 13.

- 25 a) p - Nitrobenzyl 7 - [2 - (2 - thienyl)acetamidol] - 3 - methylsulfonyloxy - 3-cephem-4-carboxylate.

To a solution of 4.75 g. (10 mmole) of p-nitrobenzyl 7-[2-(2-thienyl)-acetamidol]-3-hydroxy-3-cephem-4-carboxylate in 50 ml. of dry dimethylacetamide were added 2 ml. of propylene oxide. To the solution was added with stirring one equivalent of methanesulfonyl chloride and stirring was continued for 3 hours. The reaction mixture was then taken up in ethyl acetate and the solution was washed with a saturated solution of sodium chloride. The washed organic phase was evaporated *in vacuo* to dryness to obtain the reaction product mixture as a residue. The reaction product was purified by preparative thin layer chromatography on silica gel using for elution 65% ethyl acetate/hexane.

The purified title compound gave the following percent elemental composition on microanalysis.

Calculated for $C_{21}H_{19}N_3O_6S_2$

Theory: C, 45.56; H, 3.46; N, 7.59; S, 17.38.

Found: C, 45.74; H, 3.56; N, 7.30; S, 17.06.

The nuclear magnetic resonance spectrum and the infrared absorption spectrum were in agreement with the structure of the product formed.

- 45 b) p-Nitro 7-[2-(2-thienyl)acetamidol]-3-fluoro-3-cephem-4-carboxylate

To 93 mg. of dicyclohexyl-18-crown-6-ether in 15 ml. of acetonitrile dried over molecular sieves were added 25 mg. of potassium fluoride which had been dried *in vacuo* at 90°C. The mixture was stirred for ten minutes, and 138 mg. of p - nitrobenzyl 7 - [2 - (2 - thienyl)acetamidol]-3-methylsulfonyloxy-3-cephem-4-carboxylate in 4 ml. of acetonitrile were then added. The mixture was stirred for 1 hour. The mixture was acidified by addition of dilute (5%) HCl, and the resulting acidified mixture was extracted with ethyl acetate. Pure product was obtained from the ethyl acetate extract by preparative thin layer chromatography on silica gel using ethyl acetate: benzene (1:1). Ten mg. of p-nitrobenzyl 7-[2-(2-thienyl)-acetamidol]-3-fluoro-3-cephem-4-carboxylate were obtained

I.R.: absorption peaks at 1792, 1740, and 1685 cm^{-1}

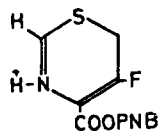
- 55 NMR ($CDCl_3$) showed signals at 6.15 (s, 2H, $\alpha-CH_2$), 4.97 (d, 1H, C_6-H), 4.20 (q, 1H, C_7-H), 3.52 (d, 1H, C_7NH), 2.32—1.7 (m, 2H, C_7-H_2) tau.

Fluorine NMR — (d, J = 10Hz).

M.S.: Calculated — 477.0465

Found — 477.0455

Fragment



Calculated — 287.0345
Found — 297.0344

Example 14.

7-[2-(2-thienyl)acetamido]-3-fluoro-3-cephem-4-carboxylic acid.

To 110 ml. of methanol and 83 mg. of pre-reduced 5 percent palladium on carbon were added 83 mg. of *p*-nitrobenzyl 7-[2-(2-thienyl)acetamido]-3-fluoro-3-cephem-4-carboxylate. The mixture was hydrogenated at 53 psig. for one hour. The resulting mixture was filtered, and the filtered catalyst was washed with methanol, and the methanol washings were added to the filtrate. The filtrate was then evaporated *in vacuo*. The residue was dissolved in ethyl acetate, and the ethyl acetate solution was extracted with dilute aqueous sodium bicarbonate. The sodium bicarbonate solution was washed with ethyl acetate, layered with ethyl acetate, and dilute aqueous HCl was added. The layered ethyl acetate was separated and evaporated to recover 7-[2-(2-thienyl)acetamido]-3-fluoro-3-cephem-4-carboxylic acid, shown by bioautogram to be biologically active.

Example 15.

p-Nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate hydrochloride

To a solution of 500 mg. of *p*-nitrobenzyl 7-[2-(2-thienyl)acetamido]-3-chloro-3-cephem-4-carboxylate in 6 ml. of methylene chloride was added 95 mg. of dry pyridine and 237 mg. of phosphorus pentachloride. The reaction mixture was stirred at room temperature for 1.5 hours, was thereafter cooled in an ice-water bath to about 5°C. and 0.6 ml. of isobutyl alcohol were added. On continued cooling and stirring the reaction product, *p*-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate hydrochloride, crystallized from the reaction mixture. The product was filtered, washed with cold methylene chloride and dried to yield 200 mg. of the crystalline product melting with decomposition at about 168°C.

Percent elemental composition for $C_{14}H_{13}ClN_3O_5S \cdot HCl$:

Theory: C, 41.39; H, 3.20; N, 10.34; Cl, 17.45

Found: C, 41.14; H, 3.31; N, 10.44; Cl, 17.29.

I.R. (nujol mull): showed absorption bands at 5.55 (β -lactam carbonyl) and at 5.78 (ester carbonyl) microns.

UV (pH 7 buffer): showed absorption maximum

λ max 268 m μ ($\epsilon=13,800$)

N.M.R. (DMSO- d_6): signals at 5.97 (s, 2H, C_2-H_2), 4.8—4.5 (m, 4H, C_6-H and ester CH_2), and 2.35—1.6 (q, 4H, aromatic H) tau.

Example 16.

7-Amino-3-chloro-3-cephem-4-carboxylic acid

To a solution of 750 mg. (1.85 mmole) of *p*-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate hydrochloride in 20 ml. of tetrahydrofuran and 40 ml. of methanol was added a suspension of 750 mg. of pre-reduced 5% palladium on carbon catalyst in 20 ml. of ethanol and the suspension was hydrogenated under 50 psi of hydrogen at room temperature for 45 minutes. The catalyst was filtered and washed with THF and water. The filtrate and catalyst washes were combined and evaporated to dryness. The residue was dissolved in a water-ethyl acetate mixture and the pH adjusted to pH 3. The insoluble product was filtered and triturated with acetone. The product was then dried to yield 115 mg. of 7-amino-3-chloro-3-cephem-4-carboxylic acid.

I.R. (mull): absorption peaks at 5.61 (β -lactam carbonyl), and 6.2 (carboxylic acid).

NMR (D $_2$ O—NaHCO $_3$): signals at 6.25 (ABq, 2H, C_2-H_2), 4.88 (d, 1H, C_6-H) and 4.54 (d, 1H, C_7-H) tau.

U.V. (pH 7 buffer): absorption maximum at

λ max 265 m μ , $\epsilon = 7550$

Example 17.

Diphenylmethyl 7-amino-3-chloro-3-cephem-4-carboxylate

To a solution of 525 mg. of diphenylmethyl 7-[2-(2-thienyl)acetamido]-3-chloro-3-cephem-4-carboxylate in 20 ml. of methylene chloride was added 0.1 ml. of dry pyridine and 237 mg. of phosphorus pentachloride. The reaction mixture was stirred for 2 hours at room temperature and was then cooled in an ice-water mixture. To the cold mixture was added 0.6 ml. of isobutanol and after 30 minutes the reaction mixture was evaporated. The residue was dissolved in ethyl acetate and the solution was washed with 5% sodium bicarbonate and with water and was dried. The dried solution was evaporated to dryness and the residue was triturated with ether to yield 190 mg. of 3-chloro nucleus ester, diphenylmethyl 7-amino-3-chloro-3-cephem-4-carboxylate.

I.R. (mull): absorption peaks at 5.7 and 5.9 (β -lactam and ester carbonyl) microns.

NMR (CDCl_3): signals at 6.35 (ABq, 2H, $\text{C}_2\text{--H}_2$), 4.78 (2d, 2H, $\text{C}_6\text{--H}$ and $\text{C}_7\text{--H}$), 3.05 (s, 1H, ester CH) and 2.65 (s, 10H, aromatic H).

Example 18.

Following the 7-acyl side chain cleavage reaction conditions described in Example 17 p-nitrobenzyl 7-amino-3-fluoro-3-cephem-4-carboxylate is prepared from the intermediate of Example 13, p-nitrobenzyl 7-[2-(2-thienyl)acetamido]-3-fluoro-3-cephem-4-carboxylate.

Example 19.

Following the 7-acyl side chain cleavage reaction procedure described by Example 15 diphenylmethyl 7-amino-3-bromo-3-cephem-4-carboxylate is prepared from diphenylmethyl 7-phenoxyacetamido-3-bromo-3-cephem-4-carboxylate.

Example 20.

7-(D- α -Phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid

To a suspension of 280 mg. (1.2 mmole) of 7-amino-3-chloro-3-cephem-4-carboxylic acid in 14 ml. of acetonitrile was added with stirring at room temperature 0.5 ml. of N,O-bis-(trimethylsilyl)acetamide to form the soluble disilylmethyl derivative thereof. The solution was cooled to 0°C. and was slowly added to a solution of the mixed anhydride formed by reacting 408 mg. (1.5 mmole) of methyl 3- α -carboxybenzylaminocrotonate sodium salt with 161 mg. (1.7 mmole) of methyl chloroformate in the presence of 2 drops of N,N-dimethylbenzyl amine in 7 ml. of acetonitrile. The mixture was stirred at ice bath temperature for 2 hours, 1 ml. of methanol was added and the mixture was filtered to remove insoluble impurities. To milliliters of water were added to the filtrate and the pH was adjusted momentarily to pH 1.5, to effect removal of the enamine block, and then to pH 4.5 with triethylamine. After stirring for an additional hour at ice-bath temperature the reaction product, 7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid (zwitterion) precipitated from the reaction mixture as a crystalline solid. The product was filtered, was washed with acetonitrile and dried in vacuo to yield 200 mg.

The product has the following physical characteristics:

Elemental analysis for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_4\text{SCl}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$:

Theory: C, 47.80; H, 4.01; N, 11.15; Cl, 9.40%

Found: C, 47.55; H, 4.12; N, 10.98; Cl, 9.21%

I.R. Spectrum (mull): absorption peaks at 2.9 (amide NH), 5.70 (β -lactam carbonyl) and 6.28 (carboxylate) microns.

NMR Spectrum ($\text{D}_2\text{O}/\text{DCl}$): signals at 6.5–6.7 (ABq, 2H, $\text{C}_2\text{--H}_2$), 4.84 (d, 1H, $\text{C}_6\text{--H}$), 4.26 (d, 1H, $\text{C}_7\text{--H}$) and 2.44 (s, 5H, aromatic H) tau.

UV Spectrum (pH 7 buffer):

λ max 265 m μ (ϵ =6,800).

Example 21.

To a solution of 500 mg. (1.85 mmole) of methyl 3- α -carboxybenzylaminocrotonate sodium salt (formed with phenylglycine and methyl acetoacetate) in 20 ml. of acetonitrile was added 4 drops of N,N-dimethylbenzylamine and the

solution was cooled in a dry ice-carbon tetrachloride mixture with stirring. To the cold solution was slowly added 184 mg. (1.95 mmole) of methyl chloroformate to form the mixed anhydride. After 20 minutes a pre-cooled solution of 750 mg. (1.85 mmole) of *p*-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate and 188 mg. (1.85 mmole) of triethylamine in 40 ml. of acetone was added. The addition took place over 3 minutes and thereafter the reaction mixture was stirred in the cold for 30 minutes and then at room temperature for 2 hours. The reaction mixture was filtered to remove insoluble impurities and was evaporated in vacuo. The reaction product residue was dissolved in a mixture of ethyl acetate-water and the pH of the solution was adjusted to pH 7. The organic layer was separated and washed with water. After drying over magnesium sulfate the organic layer was concentrated under vacuum to a small volume. Upon addition of *n*-hexane to the concentrate, 620 mg. of *p*-nitrobenzyl 7-[N-(1-carbomethoxy-2-propenyl)-D- α -phenylglycylamido]-3-chloro-3-cephem-4-carboxylate precipitated from the reaction mixture.

The product gave the following elemental analysis and nuclear magnetic resonance spectrum:

Elemental analysis for $C_{27}H_{26}N_4O_8SCl$:

Theory: C, 53.87; H, 4.35; N, 9.31%.

Found: C, 54.05; H, 4.13; N, 9.36%.

NMR (DMSO d_6): signals at 8.20 (s, 3H, enamine CH_3), 6.60 (ABq, 2H, C_2-H_2), 6.45 (s, 3H, ester CH_3), 5.48 (s, 1H, enamine vinyl H), 4.90-4.1 (m, 5H, C_6-H , C_7-H , $\alpha-CH$ and ester CH_2) and 3.10-1.5 (m, 9H, aromatic H) tau.

The product, 540 mg. (0.9 mmole) was dissolved in 40 ml. of acetonitrile containing 20 ml. of water and the solution was first cooled in an ice-water mixture and then acidified momentarily to pH 1.5 and thereafter adjusted to pH 2.5. The mixture was evaporated and the residue was dissolved in 40 ml. of tetrahydrofuran and 80 ml. of methanol.

To the solution was added 540 mg. of 5% palladium on carbon (pre-reduced in 20 ml. of ethanol for 45 min. under 50 psi hydrogen at room temperature) and the solution was hydrogenated at room temperature for 2.5 hr. under 50 psi hydrogen pressure. The catalyst was filtered and was washed on the filter with methanol, THF and with water. The filtrate and washes were combined and evaporated to dryness in vacuo. The reaction product residue was dissolved in water-ethyl acetate and the pH of the solution was adjusted to pH 4.5. The aqueous phase was separated, was washed with ethyl acetate and was evaporated to a small volume of about 2 ml. On cooling, 65 mg. of the product, 7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid, precipitated as a crystalline solid from the cold concentrate.

Example 22.

To a suspension of 3.0 g. (8.1 mmole) of *p*-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate in 200 ml. of tetrahydrofuran (dried with molecular sieve) were added 2.1 g. (8.3 mmole) of N-(*t*-butoxycarbonyl) D- α -phenylglycine and 2.0 g. (8.3 mmole) of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). The reaction mixture was stirred overnight at room temperature and the solvent was evaporated under reduced pressure. The residue was dissolved in a mixture of ethyl acetate and water and the organic phase was separated. The organic phase was cooled and successively washed with a cold 5 percent aqueous solution of sodium bicarbonate, a cold solution of 5 percent hydrochloric acid, and then with water. The washed solution was dried over magnesium sulfate, filtered, and concentrated by evaporation under reduced pressure to a volume of about 50 ml. From the concentrate, 3.7 g. (63 percent yield) of the reaction product, *p*-nitrobenzyl 7-[D-2-(*t*-butoxycarbamido)-2-phenylacetamido]-3-chloro-3-cephem-4-carboxylate, was obtained as a crystalline product. A second crop of the product weighing about 2 g. was obtained by further concentration of the filtrate obtained after filtering the first crop material.

Elemental analysis calculated for $C_{37}H_{37}ClN_4O_8S$:

Theory: C, 53.78; H, 4.51; N, 9.29.

Found: C, 52.66; H, 4.36; N, 8.88.

U.V. (acetonitrile) showed a maximum at 268 $m\mu$ ($\epsilon=17,100$).

I.R. The following significant absorption peaks were observed in the infrared spectra of the product. Amide NH absorption peak at 3.05 μ and carbonyl absorption peaks at 5.59, 5.75, and 6.0 μ .

5 NMR (CDCl_3): signals at 8.60 (s, 9H, t-BOC), 6.45 (ABq, 2H, $\text{C}_2\text{--H}_2$), 5.03 (d, 1H, $\text{C}_6\text{--H}$), 4.67 (s, 3H, α CH and ester CH_3), 4.12 (m, 3H, $\text{C}_7\text{--H}$ and amide NH), and 2.72–1.74 (m, 10H, aromatic H and amide NH) tau. 5

10 To a solution of 3.0 g. (5.0 mmole) of the product in 15 ml. of dry tetrahydrofuran (dried with molecular sieve) and 185 ml. of methanol were added 3 g. of pre-reduced 5 percent palladium on carbon. The catalyst was pre-reduced in ethanol for 30 minutes at room temperature under a hydrogen pressure of 50 psi. Following the addition of the pre-reduced catalyst, the product was hydrogenated at room temperature for one hour under a hydrogen pressure of 50 psi. The catalyst was filtered and washed on the filter with tetrahydrofuran and methanol. The filtrate and washes were combined and evaporated under reduced pressure. The residue was dissolved in ethyl acetate and water was added. The pH of the mixture was adjusted to pH 7 by the addition of 1N sodium hydroxide. The aqueous phase was separated and was washed with ethyl acetate. The aqueous phase was then layered with ethyl acetate and back titrated to pH 2.5 with 1N hydrochloric acid. The organic phase was separated from the aqueous phase and was washed with water and dried over magnesium sulfate. The dried organic phase was evaporated under reduced pressure to dryness to obtain the reaction product, 7-[D-2-(t-butyloxycarbamido)-2-phenylacetamido]-3-chloro-3-cephem-4-carboxylic acid, as a dry solid residue. The product was crystallized from 70 ml. of ether containing 20 ml. of petroleum ether to yield 1.75 g. (75 percent) of crystalline product. 25

Elemental analysis calculated for $\text{C}_{20}\text{H}_{22}\text{ClN}_3\text{O}_6\text{S}$:

Theory: C, 51.34; H, 4.74; N, 8.98; Cl, 7.58.

Found: C, 51.02; H, 4.96; N, 8.75; Cl, 7.30.

U.V. (acetonitrile) maximum at 268 μ ($\epsilon=7400$).

30 NMR (CDCl_3): signals at 8.55 (s, 9H, t-BOC), 6.48 (ABq, 2H $\text{C}_2\text{--H}_2$), 5.0 (d, 1H, $\text{C}_6\text{--H}$), 4.63 (d, 1H, $\alpha\text{-CH}$), 4.25 (q, 1H, $\text{C}_7\text{--H}$), 3.90 (d, 1H, amide NH), and 2.59 (s, 5H, aromatic H) tau. 30

35 To a solution of 420 mg. (2.2 mmole) of p-toluene-sulfonic acid in 5 ml. of acetonitrile were added 468 mg. (1 mmole) of the crystalline hydrogenolysis product. The solution was allowed to stand at room temperature for about 16 hours and was then diluted with 0.5 ml. of water. The pH of the solution was adjusted to pH 4.8 at which 320 mg. (87 percent) of the crystalline deblocked product, 7-(D-phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid, precipitated from the solution. The product was collected by filtration and dried. 35

40 Elemental analysis calculated for $\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S} \cdot \text{H}_2\text{O}$: 40

Theory: C, 47.80; H, 4.01; N, 11.15; Cl, 9.40.

Found: C, 48.04; H, 3.82; N, 11.18; Cl, 9.70.

Example 23.

45 The antibiotic compound 7 - (D - α - phenylglycylamido) - 3 - chloro - 3-cephem-4-carboxylic acid, was also prepared from p-nitrobenzyl 7 - [D - 2 - (t-butyloxycarbamido) - 2 - phenylacetamido] - 3 - chloro-3-cephem-4-carboxylate by a deblocking method alternative to that described in the preceding example. In this method, the t-butyloxycarbonyl amino protecting group is removed first and thereafter the p-nitrobenzyl ester group is cleaved with zinc and hydrochloric acid in dimethylformamide. This alternative preparation is described in the following paragraphs. 50

55 To a solution of 2.4 g. (12.6 mmole) of p-toluene-sulfonic acid in 60 ml. of acetonitrile was added 3.6 g. (6 mmole) of p-nitrobenzyl 7-[D-2-(t-butyloxycarbamido)-2-phenylacetamido]-3-chloro-3-cephem-4-carboxylate. The reaction solution was stirred at room temperature for about 15 hours during which time the amino deblocked product, p-nitrobenzyl 7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylate, as the p-toluenesulfonic acid salt, formed a crystalline precipitate. 55

60 The product was filtered, washed with acetonitrile and vacuum dried. Yield 3.1 g. (81 percent). 60

ed yl			Elemental analysis calculated for $C_{29}H_{27}ClN_4O_6S_2$: Theory: C, 51.58; H, 4.06; N, 8.29, Cl, 5.25. Found: C, 51.51; H, 4.14; N, 8.12, Cl, 5.60.	
id. id	5	5	Infrared absorption spectrum (chloroform): carbonyl absorption peaks at 5.61, 5.80, and 5.95 microns. Tosylate salt absorption peak at 6.29 microns.	5
ry ed ed of et re o- er as N yl a- d- m to l- re m	10	10	Nuclear magnetic resonance spectrum (DMSO d_6): signals at 7.70 (s, 3H, p-toluenesulfonic acid salt- CH_3), 6.61 (s, 3H, side chain NH_2), 6.20 (ABq, 2H, C_2-H_2), 4.94 (broad singlet, 1H, $\alpha-CH$), 4.80 (d, 1H, C_6-H), 4.51 (s, 2H, ester CH_2), 4.08 (q, 1H, C_7-H), 2.95—1.62 (m, 14H aromatic hydrogen and amide NH), and 0.32 (d, 1H, amide NH).	10
	15	15	The amino deblocked p-toluenesulfonic acid salt product obtained as described above was deesterified to obtain the antibiotic compound as follows. A solution of 1.5 g. (2.2 mmole) of the amine deblocked p-toluenesulfonic acid salt product obtained above in 10 ml. of dry dimethylformamide (dried with molecular sieve) was cooled in an ice-alcohol bath. To the cold solution was added 2 ml. of concentrated hydrochloric acid. Zinc dust, 400 mg. (6.1 mmole) was added to the cold solution portionwise over a period of about 15 minutes. The reaction mixture was stirred in the cold for 30 minutes and was then allowed to warm to room temperature with continued stirring. The reaction mixture was stirred at room temperature for about one hour and was filtered. The pH of the filtrate was adjusted to 6.8 with triethylamine. The product, 7-(D- α -phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid as the bis-dimethylformamide solvate of the zwitterion, formed as a white crystalline precipitate. The product was filtered, washed with 10 ml. of cold dimethylformamide and then with 6 ml. of diethyl ether. The washed product was vacuum dried to yield 800 mg. (71 percent).	15
	20	20	Elemental analysis calculated for $C_{21}H_{26}ClN_3O_6S \cdot 2DMF$ Theory: C, 49.07; H, 5.49; N, 14.63; Cl, 6.90. Found: C, 48.84; H, 5.53; N, 13.48; Cl, 7.18.	20
	25	25	Ultraviolet absorption spectrum (acetonitrile): maximum at 265 $m\mu$ ($\epsilon=6,000$). Electrometric titration (66 percent aqueous DMF): pK_a at 4.55 and 7.2.	25
.0 e	30	30	Nuclear magnetic resonance spectrum (D_2O/DCI): signals at 6.34 (2s, 6H, $DMF-CH_3$), 6.33 (ABq, 2H, C_2-H_2), 4.85 (d, 1H, C_6-H), 4.64 (s, 1H, $\alpha-CH$), 4.27 (d, 1H, C_7-H), 2.41 (s, 5H aromatic H), and 1.84 (s, 2H, $DMF-(H)$ tau).	30
l. is 6 is d 2-	35	35	Example 24. 7-[D- α -(3-hydroxyphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid	35
	40	40	N-(t-Butyloxycarbonyl) D-3-hydroxyphenylglycine, 2.9 g. (11 mmole) was reacted with 3.7 g. (10 mmole) of p-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate and 2.6 g. (10.5 mmole) of the coupling reagent EEDQ. The reaction was carried out and the product isolated by following the procedures described in the acylation process of Example 22. The product (see below) was obtained as an amorphous solid after trituration with ether to yield 2.8 g. (46 percent).	40
	45	45	Elemental analysis calculated for $C_{27}H_{27}ClN_4O_9S$: Theory: C, 52.39; H, 4.40; N, 9.05. Found: C, 52.16; H, 4.59; N, 8.79.	45
l. y n d e e	50	50	Ultraviolet absorption spectrum (acetonitrile): maximum at 270 $m\mu$ ($\epsilon=17,200$). Nuclear magnetic resonance spectrum ($CDCl_3$): signals at 8.59 (s, 9H, t-BOC), 6.50 (ABq, 2H, C_2-H_2), 5.06 (d, 1H, C_6-H), 4.66 (s, 1H, $\alpha-CH$), 4.09 (m, 2H, 2_7-H), 3.34—1.70 (m, 9H, aromatic H and amide NH) tau.	50
f - n e e 2	55	55	The product, p-nitrobenzyl 7 - [D - 2 - (t - butyloxycarbamido) - 2 - (3-hydroxyphenyl) - acetamido] - 3 - chloro-3-cephem-4-carboxylate, 3.5 g. of (5.6 mmole), was hydrogenated in ethanol in the presence of pre-reduced 5 percent palladium on carbon by following the deesterification procedure described in Example 22. The product, 7-[D-2-(t-butyloxycarbamido)-2-(3-hydroxyphenyl)-acetamido]-3-chloro-3-cephem-4-carboxylic acid, was obtained crystalline by trituration of the amorphous crude product with a solution of n-hexane in diethyl ether. Yield: 1.5 g. (55 percent).	55
	60	60		60

Ultraviolet absorption spectrum (acetonitrile):
maximum at 272 m μ ($\epsilon=8,280$).
Electrometric titration (66 percent aqueous DMF):
pKa at 4.5.

- 5 The above product, 1.3 g. (2.7 mmole) was reacted with 1.1 g. (5.9 mmole) of p-toluenesulfonic acid in 28 ml. of acetonitrile to effect the removal of the t-butyloxycarbonyl protecting group. The procedure was carried out essentially according to the method described in Example 22. 5
- 10 The product, 7-[D- α -(3-hydroxyphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid, crystallized from the reaction mixture, was filtered and then vacuum dried. Yield, 700 mg. (64 percent). 10

Elemental analysis calculated for $C_{15}H_{14}ClN_3O_5S \cdot 1H_2O$:

Theory: C, 44.83; H, 4.01; N, 10.46.

Found: C, 45.12; H, 4.06; N, 10.31.

- 15 Ultraviolet absorption spectrum (pH 6 buffer): 15
maximum at 268 m μ ($\epsilon=9,750$).
Nuclear magnetic resonance spectrum (D_2O/DCI): signals at 6.31 (ABq, 2H, C_2-H), 4.81 (d, 1H, C_6-H), 4.52 (s, 1H, $\alpha-CH$), 4.26 (d, 1H, C_7-H), and 3.1—2.5 (m, 4H, aromatic H) tau.

- 20 Example 25. 20

7-(D-4-Chlorophenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid

- By following the procedures described in Example 22, 850 mg. (2.3 mmole) of p-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate was acylated with 700 mg. (2.5 mmole) of N-t-butyloxycarbonyl D-4-chlorophenylglycine and 567 mg. (2.3 mmole) of EEDQ to provide 1.2 g. of p-nitrobenzyl 7-[D-2-(t-butyloxycarbamido)-2-(4-chlorophenyl)acetamido]-3-chloro-3-cephem-4-carboxylate. 25

- The product, 1.2 g. (1.9 mmole) was hydrogenated at room temperature in the presence of pre-reduced 5 percent palladium on carbon to effect the removal of the p-nitrobenzyl ester group and provide 450 mg. of 7-[D-2-(t-butyloxycarbamido)-2-(4-chlorophenyl)acetamido]-3-chloro-3-cephem-4-carboxylic acid as a crystalline product. The product was obtained crystalline by trituration of the amorphous reduction product with diethyl ether. 30

Elemental analysis calculated for $C_{20}H_{21}Cl_2N_3O_6S$:

Theory: C, 47.82; H, 4.21; N, 8.36; Cl, 14.11.

Found: C, 47.75; H, 4.43; N, 8.11; Cl, 14.15.

- 35 Electrometric titration (66 percent aqueous DMF): 35
pKa at 4.4; apparent molecular weight = 508; calculated MW=502.

- The deesterification product, 450 mg. (0.9 mmole), was reacted with p-toluenesulfonic acid in acetonitrile by following the procedure described in previous examples to effect the removal of the t-butyloxycarbonyl protecting group and provide 160 mg. of crystalline 7-[D- α -(4-chlorophenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid in the zwitterionic form. 40

Elemental analysis calculated for $C_{15}H_{13}Cl_2N_3O_5S \cdot 1H_2O$:

Theory: C, 42.86; H, 3.59; N, 9.99; Cl, 16.87.

Found: C, 43.07; H, 3.63; N, 9.69; Cl, 16.75.

- 45 Ultraviolet absorption spectrum (pH 6 buffer): 45
maxima at 265 m μ ($\epsilon=8,100$) and 2.25 m μ ($\epsilon=13,900$).
Electrometric titration (66 percent aqueous DMF):
pKa = 4.15 and 6.8; apparent molecular weight = 407, calculated MW 403. 50

Example 26.

7-[D- α -(4-Hydroxyphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid

- N-(t-butyloxycarbonyl) D-4-hydroxyphenylglycine, 2.9 g. (11 mmole) was reacted with 3.7 g. (10 mmole) p-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate and 2.6 g. (10.5 mmole) of the coupling reagent EEDQ in dry tetrahydrofuran. The reaction was carried out and the product isolated by following the procedures described in the acylation process of Example 22. 55

The product, 3.7 g. (60 percent yield) was obtained crystalline from cold diethyl ether.

The product, p-nitrobenzyl 7 - [D - 2 - (t - butyloxycarbamido) - 2 - (4-hydroxyphenyl)acetamido]-3-chloro-3-cephem-4-carboxylate gave the following percent elemental analysis and nuclear magnetic resonance spectrum.

Elemental analysis calculated for $C_{27}H_{27}N_4O_9SCl$:

Theory: C, 52.39; H, 4.40; N, 9.05.

Found: C, 52.12; H, 4.26; N, 8.91.

Nuclear magnetic resonance spectrum (DMSO d_6) showed signals at 8.62 (s, 9H, t-BOC-CH₃), 6.16 (ABq, 2H, C₂-H₂), 4.81 (d, 1H, C₆-H), 4.75 (d, 1H, α -CH), 4.53 (s, 2H, ester-CH₂), 4.18 (q, 1H, C₇-H), 7.04 and 2.0 (2q, 8H, aromatic H), 0.76 (d, 1H, C₇-NH) and 0.58 (s, 1H, p-OH), tau.

The product, 2.2 g. (3.5 mmole) was hydrogenated in ethanol in the presence of pre-reduced 5 percent palladium-on-catalyst to affect the removal of the p-nitrobenzyl group. The de-esterified product, 7-[D-2-(t-butyloxycarbamido)-2-(4-hydroxy)phenyl-acetamido]-3-chloro-3-cephem-4-carboxylic acid, was obtained crystalline from a mixture of diethyl ether and n-hexane. One gram of the product was obtained.

Elemental analysis calculated for $C_{20}H_{22}ClN_3O_7S$:

Theory: C, 49.64; H, 4.58; N, 8.08.

Found: C, 48.92; H, 4.40; N, 8.24.

Nuclear magnetic resonance spectrum (DMSO d_6) showed signals at 8.61 (s, 9H, t-BOC-CH₃), 6.26 (ABq 2H, C₂H₂), 4.89 (d, 1H, C₆-H), 4.78 (d, 1H, α -CH) 4.28 (q, 1H, C₇-H), 3.06 (q, 4H, aromatic-H) and 1.20 (d, 1H, C₇-NH) tau.

The tBuOOC— protecting group was removed from the de-esterification product by reacting the product in acetonitrile with p-toluenesulfonic acid. From the 1 g. of product was obtained 330 mg (40 percent) of the final product 7 - [D - α - (4 - hydroxyphenyl) - glycylamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid having the following elemental analysis, nuclear magnetic spectrum and titration values.

Elemental analysis calculated for $C_{15}H_{14}ClN_3O_7S \cdot 1H_2O$:

Theory: C, 44.83; H, 4.01; N, 10.46.

Found: C, 44.92; H, 3.45; N, 10.63.

Electrometric titration in 66 percent aqueous DMF gave pK_a values of 4.2, 7.7 and 12.4. The apparent molecular weight calculated as 384; the calculated molecular weight is 383.8.

Nuclear magnetic spectrum (D₂O/DCI) showed signals at 6.32 (ABq, 2H, C₂-H₂), 4.84 (d, 1H, C₆-H), 4.27 (d, 1H, C₇-H), and 2.79 (q, 4H, aromatic-H) tau.

Example 27.

Following the acylation procedure and by employing the acylating reagent of Example 20, the following compounds are prepared by the acylation of the indicated 3-halo nucleus: 7 - (D - α - phenylglycylamido) - 3 - fluoro - 3 - cephem - 4 - carboxylic acid is prepared from 7 - amino - 3 - fluoro - 3 - cephem - 4 - carboxylic acid; 7 - (D - α - phenylglycylamido) - 3 - bromo - 3 - cephem - 4 - carboxylic acid is prepared from 7 - amino - 3 - bromo - 3 - cephem - 4 - carboxylic acid; and 7 - [D - α - (4 - hydroxyphenyl)glycylamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid is prepared from the enamine protected 4-hydroxy-phenylglycine mixed anhydride derivative formed with methyl chloroformate via the acylation of 7-amino-3-chloro-3-cephem-4-carboxylic acid.

Example 28.

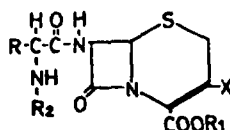
7 - [D - 2 - (2 - Thienyl)glycylamido]-3-chloro-3-cephem-4-carboxylic acid is prepared by the N - acylation of 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylic acid with 2 - thienylglycyl chloride hydrochloride in acetonitrile and in the presence of propylene oxide.

Example 29.

7 - [D - 2 - (3 - Thienyl)glycylamido] - 3 - chloro - 3 - cephem - 4 - carboxylic acid is prepared with 7 - amino - 3 - chloro - 3 - cephem - 4 - carboxylic acid via N-acylation with D-3-thienylglycyl chloride hydrochloride.

WHAT WE CLAIM IS:—

1. A compound of general formula



wherein R is phenyl, monohydroxyphenyl, dihydroxyphenyl, monohalophenyl, dihalophenyl, monomethoxyphenyl, dimethoxyphenyl, monomethylphenyl, dimethylphenyl, 2-thienyl, 3-thienyl or 2-furyl;

R₁ is hydrogen, benzyl, p-methoxybenzyl, p-nitrobenzyl, diphenylmethyl, 2,2,2-trichloroethyl, trimethylsilyl, or t-butyl;

R₂ is hydrogen or an amino-protecting group;

X is fluoro, chloro, or bromo;

or, when R₁ or R₂ is hydrogen, a pharmaceutically-acceptable, non-toxic salt thereof.

2. A compound as claimed in Claim 1, wherein R₂ is hydrogen.

3. A compound as claimed in Claim 1 or Claim 2, wherein X is chloro.

4. A compound as claimed in Claim 1, 2 or 3, wherein R is phenyl.

5. 7-(D-α-Phenylglycylamido)-3-chloro-3-cephem-4-carboxylic acid.

6. A compound as claimed in any one of Claims 1 to 3, wherein R is mono or dihydroxyphenyl.

7. 7-[D-α-(4-Hydroxyphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid.

8. 7-[D-α-(3-Hydroxyphenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid.

9. A compound as claimed in any one of Claims 1 to 3, wherein R is mono or dihalophenyl.

10. 7-[D-α-(4-Chlorophenyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid.

11. A compound as claimed in any one of Claims 1 to 3, wherein R is 2-thienyl.

12. 7-[D-α-(2-Thienyl)-glycylamido]-3-chloro-3-cephem-4-carboxylic acid.

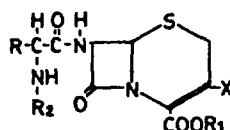
13. A compound as claimed in any one of Claims 1, 2, 4, 6, 9, and 11, wherein X is a fluorine atom.

14. 7-(D-α-Phenylglycylamido)-3-fluoro-3-cephem-4-carboxylic acid.

15. 7-[D-α-(4-Hydroxyphenyl)-glycylamido]-3-fluoro-3-cephem-4-carboxylic acid.

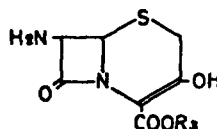
16. A compound as claimed in any one of Claims 1, 2, 4, 6, 9 and 11, wherein X is a bromine atom.

17. A process for preparing a compound of the formula



wherein R, R₁ and R₂ are as defined in any one of Claims 1, 2, 4, 6, 9 or 11, and X is chloro or bromo, which comprises

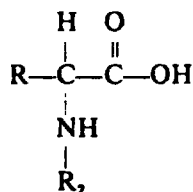
a) reacting in any sequence a 3-hydroxy-3-cephem ester of the formula



wherein R₃ is a carboxylic acid protecting ester forming group, with a chlorinating or brominating agent in a solvent capable of forming an



iminium chloride or bromide, and with an acylating reagent of the formula



- or a reactive derivative thereof, to provide the corresponding 7-(α -amino) or 7-(α -protected-amino)-acylamido-3-halo-3-cephem-4-carboxylic acid ester; and
- b) optionally removing the carboxylic acid protecting ester forming group or the amino-protecting group to provide the corresponding 7-(α -amino)acylamido-3-halo-3-cephem-4-carboxylic acid.
18. A process according to Claim 17, wherein the solvent is dimethylformamide.
19. A process according to Claim 17, or Claim 18, wherein the halogenating agent is phosphorus trichloride.
20. A process according to Claim 17 or Claim 18, wherein the halogenating agent is thionyl chloride.
21. A process according to Claim 17 or Claim 18, wherein the halogenating agent is phosphorus tribromide.
22. A process for preparing a compound according to Claim 1, substantially as hereinbefore described with particular reference to any one of Examples 20 to 29.
23. A compound according to Claim 1 substantially as hereinbefore described with particular reference to any one of Examples 20 to 29.
24. A compound according to Claim 1 whenever prepared by a method as claimed in any one of Claims 17 to 22.
25. A pharmaceutical formulation comprising a compound as claimed in any one of Claims 1 to 16, in which R_1 is hydrogen, or a pharmaceutically-acceptable salt thereof, associated with a pharmaceutically-acceptable carrier therefor.

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Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1977.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.

PATENTS ACT 1949

SPECIFICATION NO 1461323

Amendment is made in accordance with the Decision of the Principal Examiner acting for the Comptroller-General, dated the 4th day of August 1977 under Section 9 in the following manner:-

Reference has been directed in pursuance of Section 9 subsection (1) of the Patents Act 1949 to Patent Nos. 1457668 and 1462944.

THE PATENT OFFICE
16 September 1977

Bas 41476/2



* C L M S *

ERRATA

Slip No 2

SPECIFICATION NO 1361323

Page 1, line 62, *after axis delete . insert ,*

Page 3, line 50, *for ts read its*

THE PATENT OFFICE
11 October 1977

Bas 40989/1